Supplement F: Laboratory Guidance

Summary of Changes in Version 2

This version of Supplement F includes additional guidance on the types of specimens to collect for SARS-CoV testing and the optimal timing for collection. Three new appendices have been included:

- Appendix F3: Guidelines for Clinicians: The Consent Process for SARS-CoV RT-PCR and EIA Testing at CDC and Public Health Laboratories
- Appendix F6: Guidelines for Medical Surveillance of Laboratory Personnel Working with SARS-CoV
- Appendix F8: Guidelines for Laboratory Diagnosis of SARS-CoV Infection

Supplement F: Laboratory Guidance
(continued from previous page)

Contents

Supplement F: Laboratory Guidance
(continued from previous page)

Laboratory Guidance

Goals

- Provide the public health community with ready access to high-quality SARS-CoV diagnostics.
- Ensure that SARS-CoV laboratory diagnostics are used safely and appropriately and that results are interpreted appropriately.

Key concepts

- Efficient SARS-CoV diagnostic assays have been developed, but they frequently do not provide a definitive diagnosis early in illness.
- Although the sensitivity of current assays probably cannot be improved significantly, changes in the type, quality, and processing of specimens may improve the ability to detect SARS-CoV infection in patients.
- The majority of SARS-like illnesses will be caused by other respiratory pathogens; rapid and accurate diagnosis of these infections will make it easier to manage community anxiety about SARS-like illnesses.
- The possibility of false-positive and false-negative results with both PCR and serologic assays should always be considered when interpreting results; clear strategies to minimize such possibilities and to confirm test results are essential.

Priority activities

- Improve the ability to detect SARS-CoV infection by optimizing the selection and timing of specimen collection and processing.
- Provide SARS-CoV assays for RT-PCR testing to Laboratory Response Network (LRN) laboratories and for serologic testing to state public health laboratories.
- Distribute proficiency panels and questionnaires to participating laboratories to determine the ability of laboratories to provide valid SARS-CoV diagnostics.
- Provide guidance on laboratory safety for SARS-CoV and other respiratory diagnostic testing and for possible SARS-CoV-containing specimens submitted for other tests.
- Provide guidance for interpreting test results, taking into account the potential for false-positive and false-negative results and the availability of applicable clinical and epidemiologic information.
- Identify surge capacity for laboratory testing in the event of a large SARS outbreak.

Supplement F: Laboratory Guidance
(continued from previous page)

I. Rationale and Goals

Laboratory diagnostics are essential for detecting and documenting a reappearance of SARS-CoV, responding to and managing outbreaks, and managing concerns about SARS in patients with other respiratory illnesses. The identification of SARS-CoV led to the rapid development of enzyme immunoassays (EIA) and immunofluorescence assays (IFA) for serologic diagnosis and reverse-transcription PCR (RT-PCR) assays for detection of SARS-CoV RNA in clinical samples. These assays can be very sensitive and specific for detecting antibody and RNA, respectively, in the later stages of SARS-CoV infection. However, both are less sensitive for detecting infection early in illness.

As part of SARS preparedness, CDC is working to improve diagnostics by developing new tools that should make definitive diagnosis early in illness possible. In the interim, CDC is applying new knowledge about the natural history of SARS-CoV disease to improving diagnostic yield by optimizing the type, timing, and quantity of specimens collected. CDC's laboratory diagnostics plan is designed to achieve two primary goals:

- Provide ready access to high-quality SARS-CoV laboratory diagnostics for the public health community
- Ensure that SARS-CoV laboratory diagnostics are used safely and appropriately and that results are interpreted appropriately

II. Lessons Learned

The following lessons learned from the global and U.S. experience with SARS-CoV laboratory diagnostics have been considered in developing this Supplement:

- High-quality SARS-CoV diagnostic assays have been developed, but they frequently do not provide a definitive diagnosis early in illness and need to be used and interpreted carefully.
- Although the sensitivity of SARS-CoV PCR and antibody assays probably cannot be significantly improved, changes in the type, quality, and quantity of specimens and in procedures for processing specimens may improve the detection of SARS-CoV.
- The majority of SARS-like illnesses will be caused by other respiratory pathogens. Diagnosis of these infections will often make it easier to manage community anxiety about SARS-CoV.
- The possibility of false-positive and false-negative results with both PCR and serologic assays should always be considered when interpreting results. Clear strategies to minimize such possibilities and to confirm test results are essential.

III. Diagnostic Assays

Among the several types of assays used to detect SARS-CoV, RT-PCR and antibody assays are the most commonly used.

A. Real-Time RT-PCR Assays

Many laboratories have developed SARS-CoV real-time RT-PCR assays (www.cdc.gov/ncidod/sars/lab/rtpcr/), which have several advantages over traditional RT-PCR assays. Because real-time RT-PCR assays use internal probes as well as amplification primers, they can be designed to be very specific for SARS-CoV RNA (or cDNA). They can also be very sensitive, with consistent detection limits of between 1 and 10 SARS-CoV RNA copies per reaction. Real-time PCR assays can be performed faster than traditional RT-PCR assays and, because they operate as closed systems,

(continued from previous page)

with reduced risk of contamination in the laboratory. Finally, real-time RT-PCR assays can give an accurate estimate of the quantity of virus present in a sample.

As with all PCR assays, interpretation of RT-PCR tests must account for the possibility of false-negative and false-positive results. False-negative results can arise from poor sample collection or degradation of the viral RNA during shipping or storage. Application of appropriate assay controls that identify poor-quality samples can help avoid most false-negative results. A more difficult problem is the apparently low titer of SARS-CoV shed in specimens collected early in illness, which may make it difficult to confirm a diagnosis.

The most common cause of false-positive results is contamination with previously amplified DNA. The use of real-time RT-PCR helps mitigate this problem by operating as a contained system. A more difficult problem is the cross-contamination that can occur between specimens during collection, shipping, and aliquoting in the laboratory. Liberal use of negative control samples in each assay and a well-designed plan for confirmatory testing can help ensure that laboratory contamination is detected and that specimens are not inappropriately labeled as SARS-CoV positive.

In the absence of SARS-CoV transmission worldwide, the probability that a positive test result will be a "false positive" is high. To decrease the possibility of a false-positive result, testing should be limited to patients with a high index of suspicion for having SARS-CoV disease. For information on the indications for SARS-CoV testing, see *Clinical Guidance on the Identification and Evaluation of Possible SARS-CoV Disease among Persons Presenting with Community-Acquired Illness* www.cdc.gov/ncidod/sars/clinicalguidance.htm.

In addition, any positive specimen should be retested in a reference laboratory to confirm that the specimen is positive. To be confident that a positive PCR specimen indicates that the patient is infected with SARS-CoV, a second specimen should also be confirmed positive. Finally, all laboratory results should be interpreted in the context of the clinical and epidemiologic information available for the patient.

B. Antibody Assays

The most commonly used serologic assays (www.cdc.gov/ncidod/sars/lab/eia/) are based on cultured SARS-CoV antigen as either inactivated whole virus lysate for EIA or inactivated virus in cells fixed for IFA. These assays have proven to be highly specific, with no cross-reactivity with paired serum specimens from patients infected with the other known human coronaviruses (229E and OC43) or from healthy blood donors and other persons without clinical or epidemiologic evidence of SARS-CoV disease.

Antibody assays have been the most reliable indicators of SARS-CoV infection when applied to convalescent-phase serum specimens collected >28 days after onset of illness. Since previous SARS-CoV infection is still rare in most populations, demonstration of SARS-CoV-specific antibodies in a single serum specimen is sufficient for diagnosis. However, demonstration of a four-fold or greater increase in antibody titer or conversion from a negative to a positive result between acute- and convalescent-phase serum specimens provides additional confidence that SARS-CoV is linked to any corresponding illness. In some patients, antibody becomes detectable within 8 to 10 days, and most have detectable antibody by 2 weeks. However, some persons do not develop detectable antibodies until 28 days after onset of illness. Although false-positive SARS-CoV serology results are much less likely than false-positive PCR results, guidelines for confirmatory testing similar to those outlined for RT-PCR still apply. Neutralization antibody assays can also be used to detect infection. IgM assays and assays using the S or N proteins as antigens have been developed and are under evaluation.

(continued from previous page)

C. Other Assays

Among the other methods used to detect SARS-CoV are: isolation in cell culture, electron microscopy for CoV-like particles, and immunohistologic or in situ probe hybridization studies on tissue specimens. These methods are less likely to detect SARS-CoV infection than are RT-PCR or antibody assays. Although isolation of SARS-CoV in cell culture represents a definitive diagnosis, it is not recommended for routine detection as it lacks sensitivity compared to RT-PCR and also requires more restrictive Biosafety Level (BSL) 3 conditions.

Diagnostic assays for other respiratory pathogens may be helpful in differentiating SARS-CoV disease from other illnesses, but SARS patients can sometimes be infected with SARS-CoV and another respiratory pathogen simultaneously

IV. CDC's Laboratory Diagnostics Plan

CDC is planning and embarking on a range of laboratory diagnostics activities that will enhance the capacity to detect a reappearance of SARS-CoV and respond to and manage outbreaks. Objectives and descriptions of these activities are as follows.

Objective 1: Expand public health access to high-quality SARS-CoV diagnostics.

Activities

- Assay deployment -- CDC has deployed the SARS-CoV RT-PCR diagnostic assay under an Investigational Device Exemption (IDE) from the Food and Drug Administration (FDA). Protocols for both the RT-PCR and the serologic assay have been approved by CDC's Institutional Review Board (IRB). RT-PCR assays were deployed through the Laboratory Response Network (LRN) to selected laboratories in nearly all states; serologic assays have been deployed to nearly all state public health laboratories.

- Proficiency testing -- To assess the availability and quality of SARS-CoV diagnostics in laboratories that received CDC's RT-PCR and antibody assays, CDC will distribute a panel of positive and negative specimens for testing (proficiency panels). The receiving laboratories will test these specimens and send their results to CDC for analysis of findings and responses to a questionnaire. These data will provide information on the laboratory's readiness to perform SARS-CoV diagnostics (see Appendix F1).

- Assessment of SARS-CoV diagnostics in non-public health laboratories -- Determining the availability and quality of SARS-CoV testing in non-public health laboratories will provide an assessment of overall laboratory diagnostic preparedness. Several clinical pathology professional organizations conduct laboratory surveys and distribute proficiency panels. CDC will assist with SARS surveys and provide proficiency panels so that the professional organizations can assess the status of SARS-CoV diagnostics in their members' laboratories.

- Confirmatory testing -- Positive RT-PCR test results should be confirmed in a reference laboratory. Confirmatory testing is particularly important in areas with a low prevalence of SARS-CoV disease, where the positive predictive value of the assay is likely to be quite low. CDC will conduct confirmatory testing during the early phases of an outbreak. Other laboratories that are proficient in SARS-CoV diagnostics will participate in confirmatory testing as outbreaks escalate. Early in an outbreak, positive serologic tests should also be confirmed; later tests conducted in a proficient laboratory do not required confirmation.

(continued from previous page)

A key factor in the value of confirmatory RT-PCR testing is specimen handling. To interpret confirmatory test results, the aliquot of the specimen submitted for testing should not have been at risk for template contamination or degradation. The approach for and interpretation of confirmatory testing must consider all potential sources and types of template contamination (e.g., whole viral genome; genome portions; PCR products). Guidelines for confirmatory testing are provided in Appendix F2.

Objective 2: Improve the ability to detect SARS-CoV by optimizing the selection and timing of specimen collection and processing.

Most patients in the early stages of SARS-CoV disease have a low titer of virus in respiratory and other secretions and require time to mount an antibody response. In one study (in patients treated with high-dose steroids and ribavirin), nasopharyngeal (NP) aspirates were found to be PCR positive in <40% of patients during the first week of illness and in >50% of patients during the second week of illness (Peiris 2003). During the second week of illness, stool specimens were found to be PCR positive in a higher percentage of patients than were NP aspirates. Limited data suggest that serum may be the best specimen for SARS-CoV PCR diagnostics during the first few days of illness.

Activities

- Specimen collection -- CDC has developed guidance for health departments and laboratorians to maximize the efficiency and accuracy of diagnostic procedures. Clinicians and laboratorians are asked to:
  - Obtain informed consent -- A signed consent form is recommended for RT-PCR and EIA testing because neither assay has been licensed by the FDA and the RT-PCR test is being used under an FDA-approved IDE. In addition, a signed consent form is required to store specimen remainders for future investigations (see Appendix F3).
  - Collect multiple specimens -- The type and timing of specimen collection is important to maximize the probability of detecting evidence of SARS-CoV infection. Since it is not yet clear which specimen type is best for detecting viral RNA, it is important to collect different types of specimens and at multiple times during the illness. Appendix F4 provides guidance on the type and timing of specimens for SARS-CoV diagnostics.
  - Handle specimens correctly -- CDC has developed guidance for specimen collection, handling, and shipping (Appendix F4). State and local health departments can use these guidelines to educate clinicians on appropriate methods of specimen management.
- Assay sensitivity -- CDC will evaluate ways to improve assay sensitivity, such as extracting RNA from a larger volume of the specimen and including a larger amount of template RNA in the RT-PCR reaction. CDC is developing IgG and IgM assays using expressed proteins as the antigens. Preliminary data suggest that antibody assays using the SARS-CoV S protein might detect an antibody response earlier in illness.

Objective 3: Ensure that SARS-CoV specimens are handled safely and that SARS-CoV diagnostic tests are used and interpreted appropriately.

Supplement F: Laboratory Guidance
(continued from previous page)

Activities

- Biosafety guidance -- The laboratory-acquired SARS-CoV infection in Singapore (Singapore Ministry of Health 2003) and presumed laboratory-acquired SARS-CoV infection in Taiwan (Department of Health, Taiwan 2003) underscore the need to handle SARS-CoV specimens and SARS-CoV-infected tissue culture material safely. CDC has developed guidelines for handling these types of specimens and materials (Appendix F5) and for implementing a surveillance program in the event of a laboratory exposure (Appendix F6). State and local health departments can use these guidelines to educate personnel in viral diagnostic, research, and clinical laboratories about safe specimen handling and appropriate responses to a laboratory exposure.

- Test interpretation -- Clinicians should interpret SARS-CoV test results in consultation with state or local health department officials and with consideration of data on the clinical and epidemiologic features of the illness and the type and timing of specimen collection. CDC has developed guidelines to guide state and local health department staff in their consultations with clinicians about test interpretation (Appendix F7). CDC, in cooperation with CSTE, has also developed criteria for laboratory diagnosis of SARS-CoV infection (Appendix F8).

- Data reporting and integration -- State and local health departments will collect clinical and epidemiologic data on potential cases of SARS-CoV disease and report cases to CDC through a web-based reporting system. CDC will send laboratory data back to state and local health departments daily. The clinical and epidemiologic information reported to CDC and downloaded back to the states can provide a source of patient information that can help laboratorians consider appropriate testing strategies and interpret test results. With guidance from state and local health departments, CDC will facilitate access to data as requested. In addition, results of laboratory testing on any specimens submitted to CDC will be integrated into the data provided to state and local health departments, allowing timely dissemination of this information.

- Training and education – Diagnostic assays have an important role in detecting an introduction of SARS-CoV, managing a SARS outbreak, and addressing concerns about SARS. The healthcare and public health communities should be aware of the value, limitations, and appropriate use and interpretation of SARS-CoV diagnostics. CDC will provide training and educational materials that state and local health departments can use to educate clinicians and public health workers about SARS-CoV diagnostics.

- Coordination -- Coordinated information sharing among clinicians, laboratorians, and epidemiologists is central to efficient investigation of potential cases of SARS-CoV disease. CDC will assist public health laboratories and epidemiologists in developing rapid and coordinated strategies for: 1) collecting, tracking, and testing specimens, 2) interpreting test results, 3) reporting information to clinicians, and 4) communicating results to CDC, other public health officials, and the public.

Objective 4: Ensure the availability of SARS-CoV diagnostic test kits and protocols for testing other respiratory pathogens.

Activities

- Diagnostic supplies -- The supply of SARS-CoV RT-PCR and serologic test kits is limited. To ensure the availability of a sufficient number of kits to meet routine public health needs and the anticipated high demand associated with simultaneous outbreaks, CDC is monitoring both the

(continued from previous page)

deployment and number of kits. After patterns of use have been determined, CDC will plan the production of new kits to ensure that the supply can meet both projected baseline needs and the accelerated use associated with a SARS outbreak.

- Tests for alternative respiratory agents -- CDC will complete the development and initial evaluation of real-time PCR assays for the most important common respiratory pathogens in the United States and make primer and probe sequences and protocols available to the LRN and other public health laboratories.

References

Peiris JSM, Chu CM, Cheng VCC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet 2003;361:1767-72.

Singapore Ministry of Health. Biosafety and SARS incident in Singapore, September 2003: Report of the Review Panel on New SARS Case and Biosafety. Singapore: Singapore Ministry of Health; 2003 Sep. (www.moh.gov.sg/corp/sars/pdf/Report_SARS_Biosafety.pdf).

Department of Health, Taiwan. Confirmed SARS case in research laboratory in Taiwan, December 17, 2003 [news release on the Internet].Taiwan: Department of Health; 2003. (sars.doh.gov.tw/news/2003121701.html)

Supplement F: Laboratory Guidance
(continued from previous page)

Appendix F1
Proficiency Testing for Public Health Laboratories Providing SARS-CoV EIA and RT-PCR Diagnostics

CDC has developed and validated diagnostic assays for SARS-CoV, including an enzyme immunoassay (EIA) (www.cdc.gov/ncidod/sars/lab/eia/) for detection of serum antibodies to SARS-CoV and a reverse transcription-polymerase chain reaction (RT-PCR) assay (www.cdc.gov/ncidod/sars/lab/rtpcr/) for detection of SARS-CoV RNA. Both the EIA and the RT-PCR tests are sensitive and highly specific for diagnosis of SARS-CoV infection. Testing with these assays is now available through state public health laboratories and CDC's Laboratory Response Network (LRN).

CDC is implementing a quality assurance study to evaluate each laboratory's testing proficiency. To demonstrate competence in performing these tests, public health and LRN laboratories must successfully identify positive and negative specimens provided in the proficiency panels.

Process for Proficiency Panel Testing

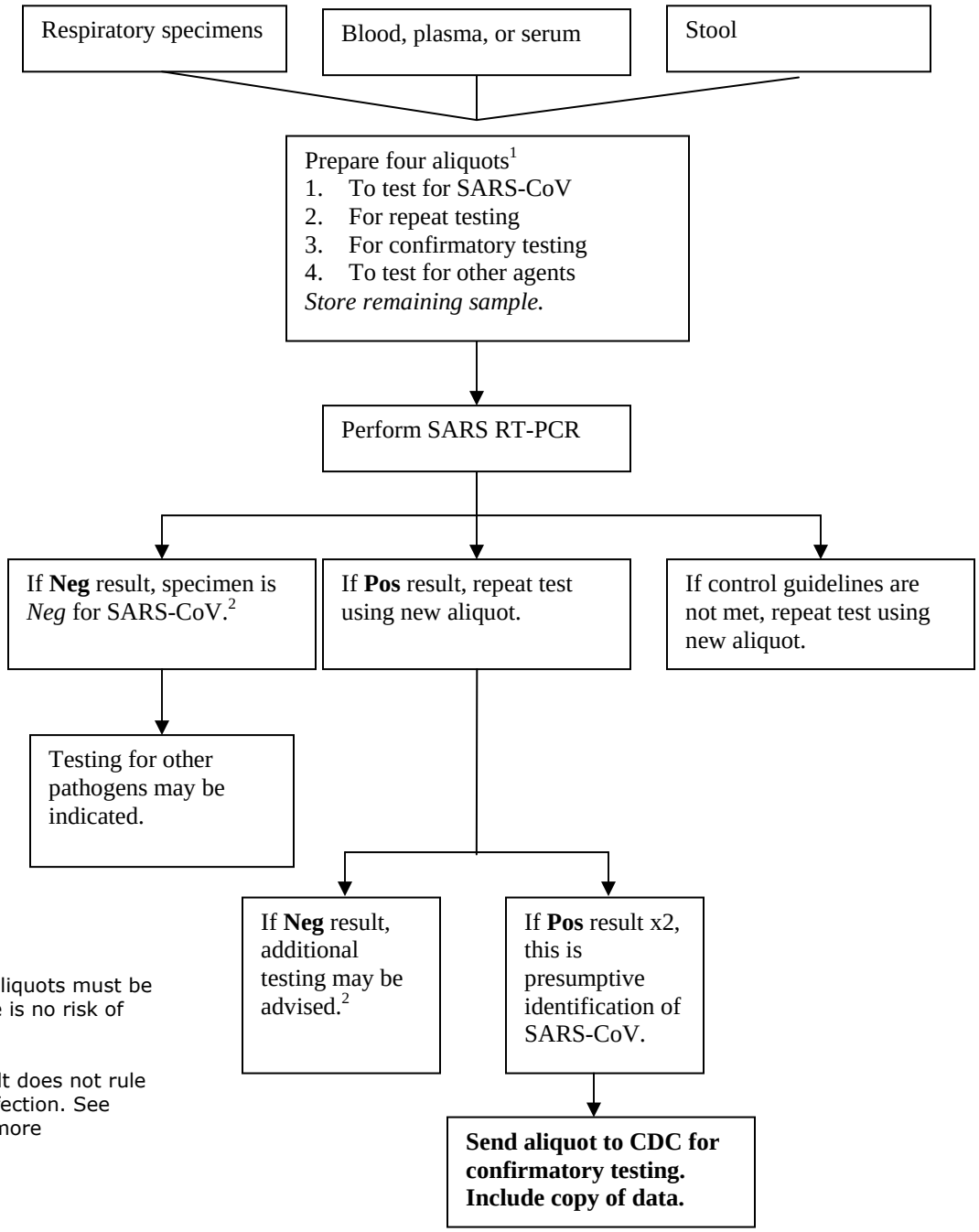
1. EIA proficiency panels will be shipped to designated public health laboratories, and RT-PCR proficiency panels will be distributed through the LRN.
2. Each laboratory should complete testing promptly and return results by the designated date.
3. Proficiency panel test results must be returned by electronic mail using a designated format.
4. Each laboratory will receive a complete summary of its own results as well as an aggregate summary of performance from all laboratories completing the proficiency testing.
5. CDC will provide a certificate of participation to the participating laboratory to help fulfill quality assurance requirements.

Results obtained from a laboratory's proficiency testing will initially be considered "educational," and laboratories will not be required to undergo additional training as remediation. However, successful completion of the proficiency test will be required for approval of a public health or LRN laboratory as a confirmatory testing site.

Supplement F: Laboratory Guidance
(continued from previous page)

Appendix F2

SARS-CoV Specimen Testing Guidelines: RT-PCR Testing

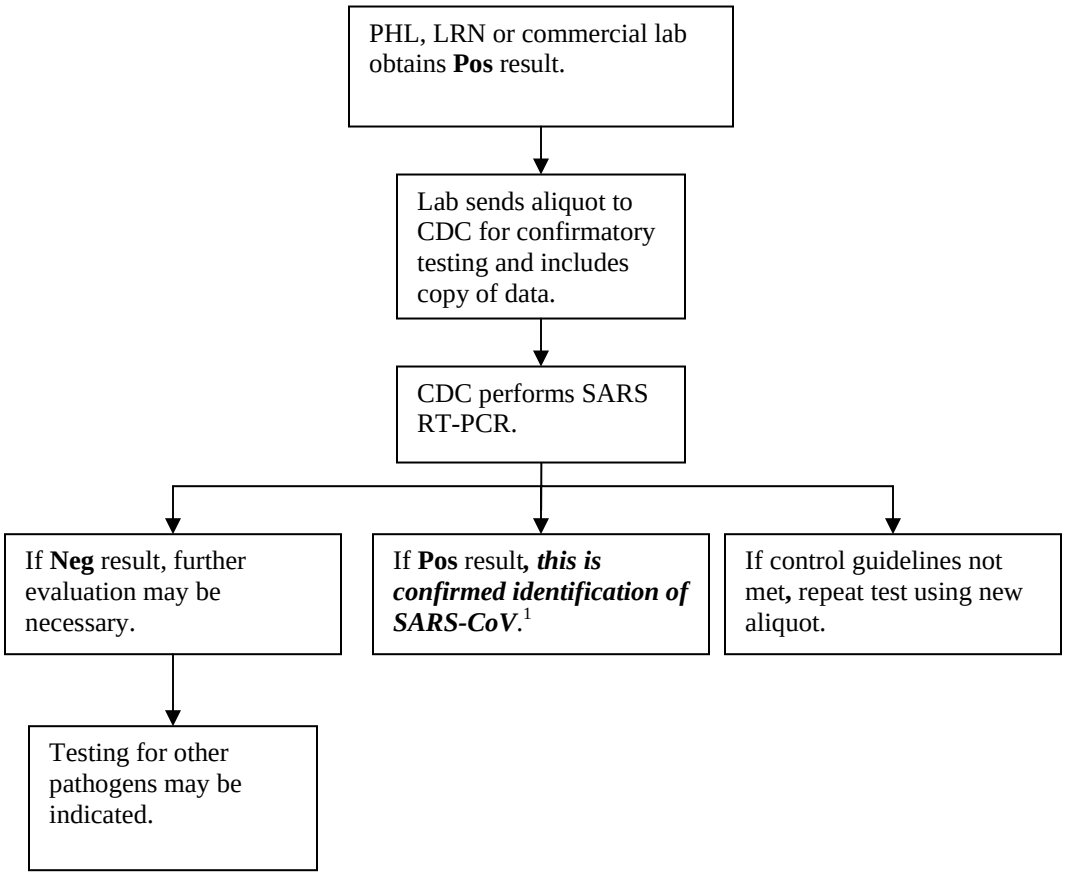


[1] Preparation of aliquots must be done where there is no risk of contamination.

[2] A negative result does not rule out SARS-CoV infection. See Appendix F7 for more information.

Supplement F: Laboratory Guidance
(continued from previous page)

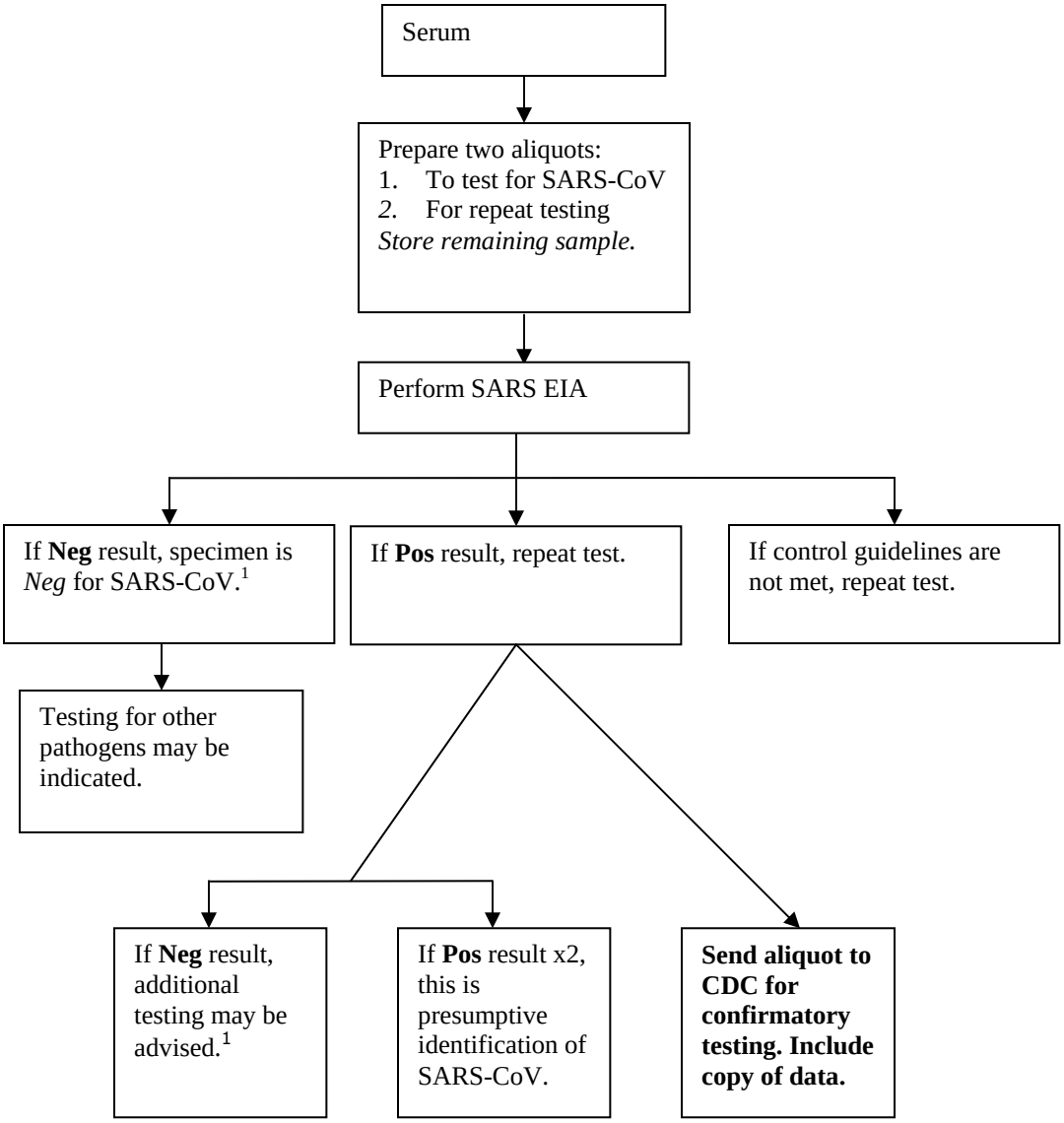
RT-PCR Confirmatory Testing



[1] Confirmed identification of SARS-CoV in a single clinical specimen is not equivalent to laboratory confirmation of SARS-CoV infection in a patient. To be confident that a PCR-positive specimen indicates infection in the patient, a second specimen should be confirmed positive. For more information on the criteria for diagnosing SARS-CoV infection, see Appendix F8.

Supplement F: Laboratory Guidance
(continued from previous page)

SARS-CoV Specimen Testing Guidelines: Serologic Testing

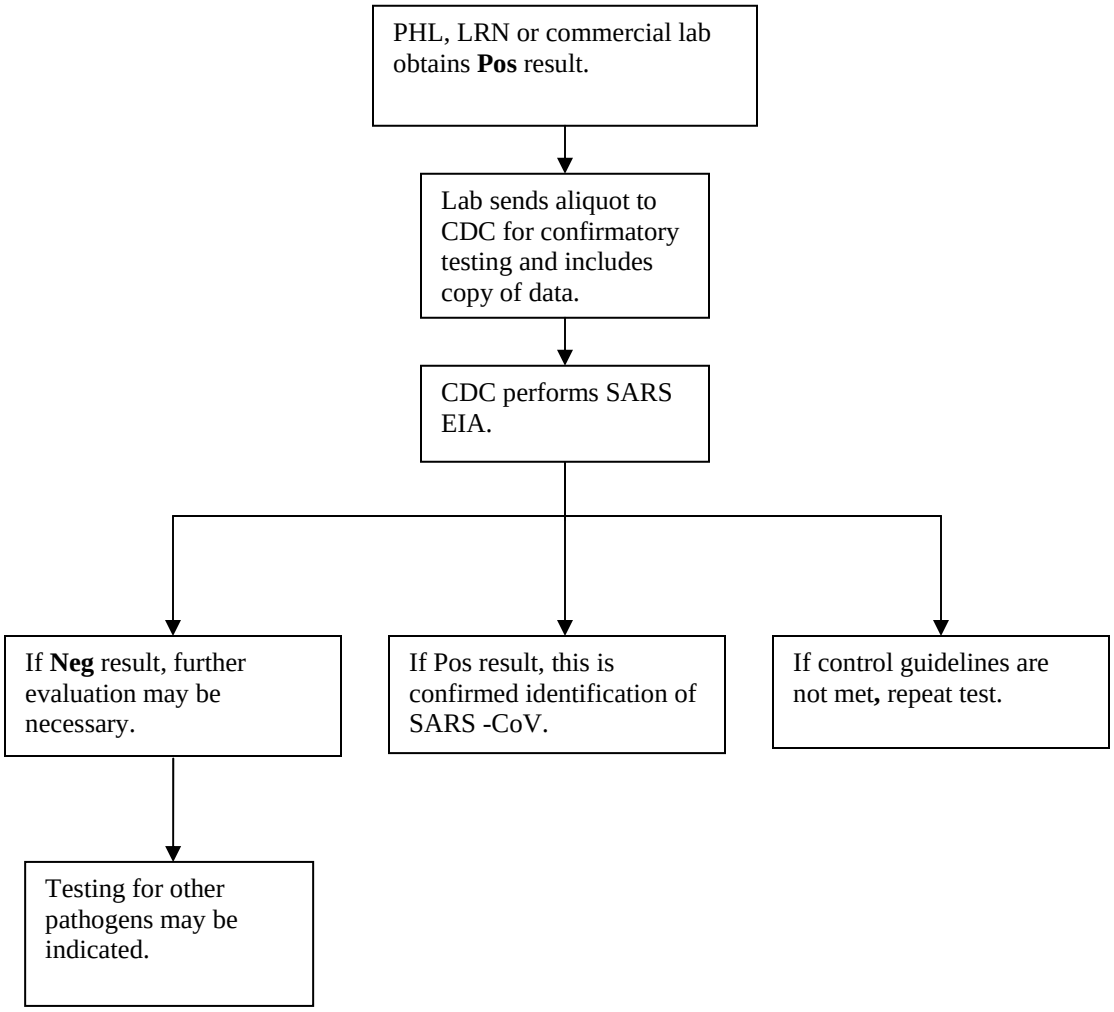


[1] A negative result does not rule out SARS-CoV infection. See Appendix F7 for more information.

Supplement F: Laboratory Guidance

(continued from previous page)

Serologic Confirmatory Testing



(continued from previous page)

Appendix F3
Guidelines for Clinicians: The Consent Process for SARS-CoV RT-PCR and EIA Testing at CDC and Public Health Laboratories

Key Messages

- A consent form is recommended when submitting specimens for SARS-CoV reverse-transcription polymerase chain reaction (RT-PCR) or enzyme immunoassay (EIA) testing.
- Due to important public health concerns, if SARS-CoV testing is indicated, specimens will be tested even if a consent form is NOT received.
- A patient information sheet, informing patients about the tests and requesting permission for long-term storage of their specimen remainders, will be sent to the physician with the patient's test results. The physician should provide this document to the patient and return a signed copy to the local or state health department if consent for long-term specimen storage is obtained.

CDC has distributed a SARS-CoV RT-PCR assay (www.cdc.gov/ncidod/sars/lab/rtpcr/) under an FDA investigational device exemption (IDE) and an EIA assay (www.cdc.gov/ncidod/sars/lab/eia/) to state and local public health laboratories to test specimens for SARS-CoV. These tests are used to evaluate persons suspected of having SARS-CoV disease. The RT-PCR assay is used to test for SARS-CoV viral RNA in respiratory samples, stool, plasma, or serum. The EIA test is used to detect SARS-CoV antibodies in blood or serum specimens. A signed consent form for performance of each test is recommended because neither test has been licensed by the FDA and the RT-PCR test is being used under an FDA-approved IDE. A signed consent form is also required for storage of specimen remainders for future investigations.

To submit specimens for SARS-CoV RT-PCR or EIA testing, healthcare providers should follow these steps:

1. **Consult the state or local health department** to determine if SARS-CoV testing is indicated.
2. **Seek informed consent** from the patient for testing.
   The RT-PCR consent form can be found at:
   www.cdc.gov/ncidod/sars/lab/rtpcr/consent.htm.
   The EIA consent form can be found at:
   www.cdc.gov/ncidod/sars/lab/eia/consent.htm
3. **Collect specimens** for testing. Guidelines for specimen collection are provided in Appendix F4.
4. **Submit specimens**, with a signed consent form and completed specimen submission form, to the state or local public health laboratory.

   Specimens will be tested at the reference public health laboratory. Final results will be reported to you through the state or local health department. Information on interpreting these test results is provided in Appendix F7.

Supplement F: Laboratory Guidance
(continued from previous page)

5. **Deliver test results** to the patient. Provide a patient information sheet/consent for long-term specimen storage to the patient along with the test results. Specimen remainders stored long term may be used for future investigations.

   The RT-PCR patient information sheet/consent for long-term specimen storage can be found at: www.cdc.gov/ncidod/sars/lab/rtpcr/participant.htm.
   The EIA patient information sheet/consent for long-term specimen storage can be found at: www.cdc.gov/ncidod/sars/lab/eia/participant.htm.

6. **If a signed patient information sheet/consent for long-term storage is obtained, fax it** to the state or local public health department. Contact information is provided at www.cdc.gov/other.htm#states.

Frequently Asked Questions

Where can I find information on how to report a possible SARS case and submit specimens for SARS-CoV testing?
This information is available through the state or local health department. Contact information is provided at: www.cdc.gov/other.htm#states.

Why is a signed informed consent form recommended for SARS-CoV testing?
A signed consent form is recommended because neither the RT-PCR test nor the EIA test has been licensed by FDA and the RT-PCR test is being used under an FDA-approved investigational device exemption (IDE). In addition, consent is required to store specimen remainders for future investigations.

Why are there two different consent forms, one for RT-PCR and one for EIA?
Two forms are required because of differing IRB review requirements; CDC's IRB reviewed and approved two separate protocols.

What happens if I submit specimens for testing without a signed consent form?
Because of the potentially serious public health impact of SARS-CoV transmission, specimens that are received by a state or local public health laboratory without a signed consent form will still be tested.

What is the patient information sheet, and when do I use it?
The patient information sheet/consent for long-term specimen storage will be sent to the physician along with the patient's test results. The physician should provide this document to the patient. It explains to the patient why SARS-CoV testing was performed on their specimens, explains what the results mean, and asks the patient for permission to store specimen remainders for future investigations.

Why should a signed patient information sheet be returned?
The patient information sheet asks the patient for permission to store specimen remainders for future investigations. If a signed consent form is not received before testing, it is necessary to receive the signed patient information sheet to store any specimen remainders. Specimens without a signed informed consent or signed patient information sheet allowing long-term specimen storage must be destroyed.

(continued from previous page)

Appendix F4
Guidelines for Collecting Specimens from Potential SARS Patients

This document updates and replaces the guidelines for specimen collection posted previously on CDC's SARS website, to reflect the most recent information on laboratory diagnostics for SARS-CoV. The main changes are as follows:[1]

- Addition of stool, serum, and plasma to the list of specimens for RT-PCR testing
- Addition of information on the optimal timing of specimen collection and testing by specimen type
- Recommendation to collect multiple specimens for RT-PCR testing

[1] Pending IRB approval.

Key Messages

- Consult the state or local health department to determine the appropriateness and details of SARS testing.
- If possible, collect multiple specimens from different body sites and at different times during illness.
- A signed consent form is recommended when collecting specimens for SARS-CoV RT-PCR or antibody testing.

Before collecting and shipping specimens for SARS-CoV testing, consult with the state health department/state epidemiologist to determine whether the patient meets the SARS case definition. Health department contact information is available at: www.cste.org/members/state_and_territorial_epi.asp.

When possible, collect multiple respiratory specimens for testing. For example, collect specimens from two different sites on the same day (e.g., one nasopharyngeal swab and a stool specimen or another respiratory specimen) or from two different times during the illness. The chart on the last page specifies recommended specimen types for SARS-CoV diagnostics by stage of illness, including postmortem specimen collection.

A signed consent form is recommended when collecting specimens for SARS-CoV RT-PCR or antibody testing. Information on the consent process for collection of respiratory specimens, blood or stool for RT-PCR testing is provided at: www.cdc.gov/ncidod/sars/lab/rtpcr/index.htm. Information on the consent process for the collection of blood/serum for antibody testing is provided at: www.cdc.gov/ncidod/sars/lab/eia/index.htm.

Supplement F: Laboratory Guidance
(continued from previous page)

RT-PCR Diagnostics

Although studies to date have not definitively determined the best specimens for SARS RT-PCR diagnostics, it is reasonable to collect:

During the first week of illness:	Nasophyaryngeal (NP) swab plus oropharyngeal (OP) swab and a serum or plasma specimen
After the first week of illness:	NP swab plus OP swab and a stool specimen

Serologic Diagnostics

Serum specimens for SARS-CoV antibody testing should be collected when the diagnosis is first suspected and at later times if indicated. An antibody response is occasionally detected during the first week of illness, likely to be detected by the end of the second week of illness, and sometimes may not be detected until >28 days after onset of symptoms.

I. Collecting Respiratory Specimens

Eight types of respiratory specimens may be collected for viral and/or bacterial diagnostics: 1) nasopharyngeal wash/aspirates, 2) nasopharyngeal swabs, 3) oropharyngeal swabs, 4) broncheoalveolar lavage, 5) tracheal aspirate, 6) pleural fluid tap, 7) sputum; and 8) post-mortem tissue. Nasopharyngeal wash/aspirates are the specimen of choice for detection of most respiratory viruses and are the preferred specimen type for children under age 2 years.

In contrast to most respiratory pathogens for which respiratory specimens are optimally collected within 72 hours after the onset of symptoms, levels of SARS-CoV may be higher later in the course of the illness.

Before collecting specimens, review the infection control precautions in Supplement I.

A. Collecting specimens from the upper respiratory tract

1. Nasopharyngeal wash/aspirate

Have the patient sit with head tilted slightly backward. Instill 1 ml-1.5 ml of nonbacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2 ml-3 ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate nasopharyngeal secretions. Repeat this procedure for the other nostril. Collect the specimens in sterile vials. Label each specimen container with the patient's ID number and the date collected. If shipping domestically, use cold packs to keep the sample at 4°C. If shipping internationally, pack in dry ice.

2. Nasopharyngeal or oropharyngeal swabs

Use only sterile dacron or rayon swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden sticks, as they may contain substances that inactivate some viruses and inhibit PCR testing.

Nasopharyngeal swabs -- Insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both nostrils.

Supplement F: Laboratory Guidance
(continued from previous page)

Oropharyngeal swabs -- Swab the posterior pharynx and tonsillar areas, avoiding the tongue.

Place the swabs immediately into sterile vials containing 2 ml of viral transport media. Break the applicator sticks off near the tip to permit tightening of the cap. Label each specimen container with the patient's ID number and the date the sample was collected. If shipping domestically, use cold packs to keep sample at 4°C. If shipping internationally, pack in dry ice.

B. Collecting specimens from the lower respiratory tract

1. Broncheoalveolar lavage, tracheal aspirate, pleural fluid tap

Centrifuge half of the specimen, and fix the cell pellet in formalin. Place the remaining unspun fluid in sterile vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®. Label each specimen container with the patient's ID number and the date the sample was collected. If shipping domestically, use cold packs to keep sample at 4°C. If shipping internationally, ship fixed cells at room temperature and unfixed cells frozen.

2. Sputum

Educate the patient about the difference between sputum and oral secretions. Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile screw-cap sputum collection cup or sterile dry container. If shipping domestically, use cold packs to keep sample at 4°C. If shipping internationally, pack in dry ice.

II. Collecting Blood Components

Serum and blood (plasma) should be collected early in the illness for RT-PCR testing. The reliability of RT-PCR testing performed on blood specimens decreases as the illness progresses.

Both acute and convalescent serum specimens should be collected for antibody testing. To confirm or rule out SARS-CoV infection, it is important to collect convalescent serum specimens >28 days after the onset of illness.

A. Collecting serum for antibody or RT-PCR testing

Collect 5 ml-10 ml of whole blood in a serum separator tube. Allow the blood to clot, centrifuge briefly, and collect all resulting sera in vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®. The minimum amount of serum preferred for each test is 200 microliters, which can easily be obtained from 5 mL of whole blood.

A minimum of 1 cc of whole blood is needed for testing of pediatric patients. If possible, collect 1 cc in an EDTA tube and in a clotting tube. If only 1cc can be obtained, use a clotting tube.

Label each specimen container with the patient's ID number and the date the specimen was collected. If unfrozen and transported domestically, ship with cold packs to keep the sample at 4°C. If frozen or transported internationally, ship on dry ice.

Supplement F: Laboratory Guidance
(continued from previous page)

B. Collecting EDTA blood (plasma) for RT-PCR

Collect 5 ml-10 ml of blood in an EDTA (purple-top) tube. Transfer to vials with external caps and internal O-ring seals. If there is no internal O-ring seal, then seal tightly with the available cap and secure with Parafilm®. Label each specimen container with patient's ID number and date of collection. Store and ship blood specimens with cold packs to keep the sample at 4°C.

III. Collecting Stool Specimens for PCR

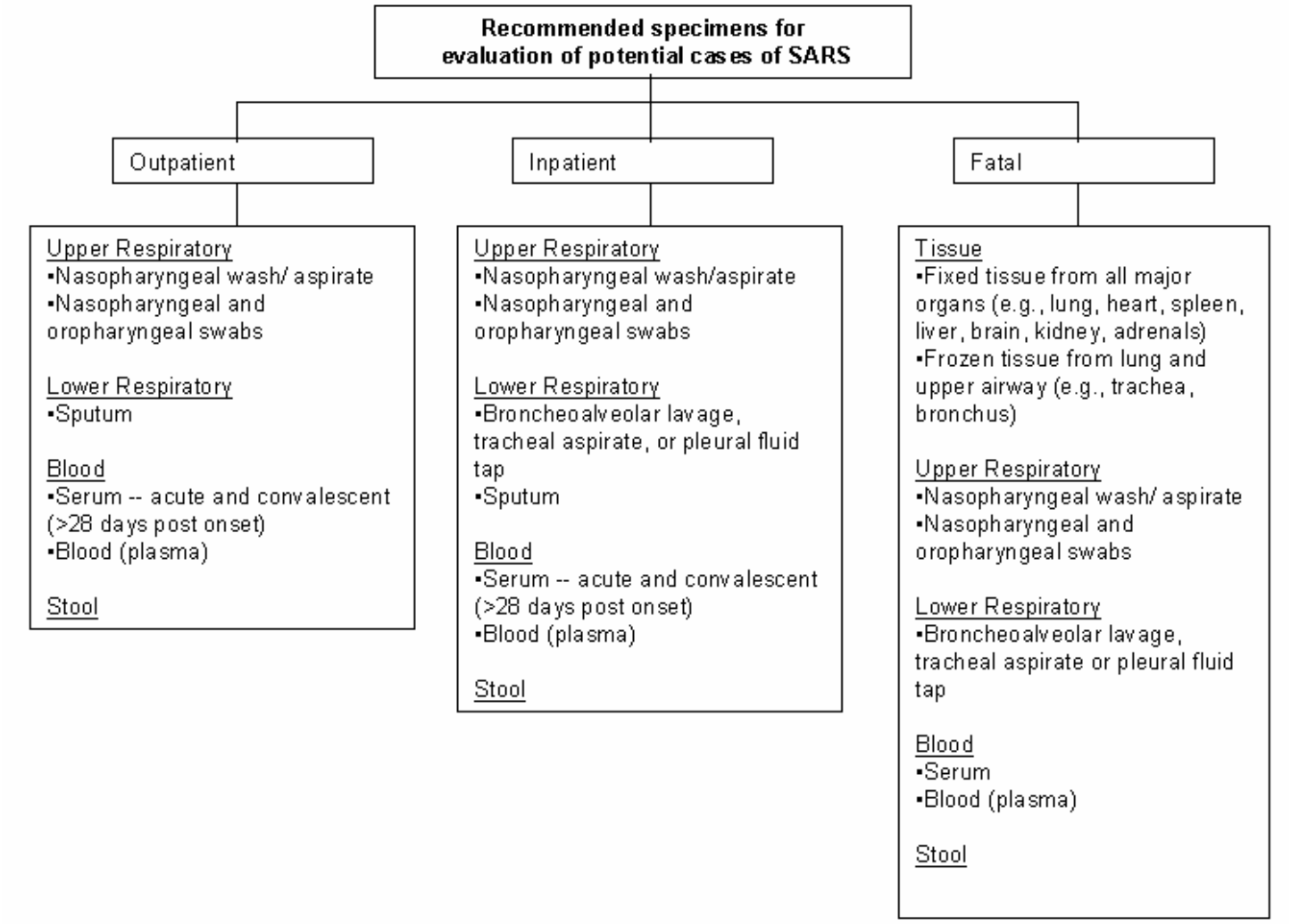
Begin collecting stool specimens as soon as possible in the course of the illness. Although collecting earlier specimens is ideal, SARS-CoV has been detected in stool as late as one month after the onset of symptoms.

Place each stool specimen -- as large a quantity as can be obtained (at least 10 cc) -- in a leak-proof, clean, dry container, and refrigerate at 4°C. Patients may drape plastic kitchen wrap across the back half of the toilet, under the toilet seat, to facilitate collection of stool specimens.

IMPORTANT: Refrigerate or freeze tubes after specimens are placed in them. If specimens will be examined within 48 hours after collection, they can be refrigerated. If specimens must be held longer than 48 hours, freeze them as soon as possible after collection. Although storage in an ultra-low freezer (-70°C) is preferable, storage in a home-type freezer (if properly set at -20°C) is acceptable for short periods.

Specimens from possible and known SARS cases must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations at (www.iata.org/dangerousgoods/index) and US DOT 49 CFR Parts 171-180 (hazmat.dot.gov/rules.htm). Step-by-step instructions on appropriate packaging and labeling are available at: www.cdc.gov/ncidod/sars/pdf/packingspecimens-sars.pdf.

Supplement F: Laboratory Guidance
(continued from previous page)



Supplement F: Laboratory Guidance
(continued from previous page)

Specimens for SARS-CoV Testing:
Priority Specimens and Timing for Collection

The likelihood of detecting infection is increased if multiple specimens, e.g., stool, serum, and respiratory tract specimens, are collected during the course of illness.

Specimen, by test type	<1 week after symptom onset	1-3 weeks after symptom onset	>3 weeks after symptom onset
RT-PCR[1] for viral RNA detection			
Sputum[2]	√[3]	√√	√
Bronchoalveolar lavage, tracheal aspirate, or pleural fluid tap[4]	√	√√	√
Nasopharyngeal wash/aspirate	√	√√	√
Nasopharyngeal and oropharyngeal swabs	√	√√	√
Serum (serum separator tube)	√√	√	not recommended
Blood (plasma) (EDTA/purple top tube for plasma)	√√	√	not recommended
Stool (minimum 10 cc specimen)	√	√√	√√
EIA[1] for antibody detection			
Serum[5] (serum separator tube)	√√	√√	√√

[1] Because of the investigational nature of both the SARS RT-PCR (reverse transcription-polymerase chain reaction) and the SARS EIA (enzyme immunoassay), it is recommended that the clinician obtain a signed informed consent form from the patient. The consent form for the RT-PCR test can be found at: www.cdc.gov/ncidod/sars/lab/rtpcr/consent.htm. The consent form for the EIA test can be found at: www.cdc.gov/ncidod/sars/lab/eia/consent.htm.

[2] A sputum specimen is preferred if the patient has a productive cough.

[3] The more checks, the better the results from a particular specimen at a specific point in the illness.

[4] Consider these specimen types if sputum is not available.

[5] Also collect a convalescent specimen >28 days post onset.

(continued from previous page)

Appendix F5
Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV

Key Messages

- Clinical laboratories performing routine hematology, urinalysis, and clinical chemistry studies, and microbiology laboratories performing diagnostic tests on serum, blood, or urine specimens should follow standard laboratory practices, including Universal Precautions, when handling potential SARS-CoV specimens. For additional information, see www.osha.gov/SLTC/bloodbornepathogens/index.html#revised_standard.
- Microbiology and pathology laboratories performing diagnostic tests on stool or respiratory specimens should handle potential SARS-CoV specimens using standard Biosafety Level (BSL)-2 work practices in a Class II biological safety cabinet.
- A detailed description of recommended facilities, practices, and protective equipment for the various laboratory biosafety levels can be found in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) manual at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm.

Although routine clinical laboratories around the world have processed an estimated several thousand diagnostic specimens from patients with SARS, no cases of SARS-CoV disease among laboratory workers performing diagnostic assays have been reported to date. However, there have been two reported cases of SARS-CoV disease in workers in research laboratories where SARS-CoV was being propagated. Until more information about the transmission of SARS-CoV in the laboratory setting is known, precautions should be taken in handling specimens (e.g., respiratory and stool specimens, unfixed lung tissue, viral cultures) that might contain large quantities of SARS-CoV.

Effective and timely communication between clinical and laboratory staff is essential to minimize the risk incurred in handling specimens from patients with possible SARS-CoV disease. Such specimens should be labeled accordingly, and the laboratory should be alerted to ensure proper specimen handling. Biosafety guidelines for handling SARS-CoV specimens, by specimen type, are provided below. Guidelines on implementing a medical surveillance system for laboratory workers are provided in Appendix F6.

A. Blood (blood, serum and plasma) and urine specimens

- Handle these specimens using Universal Precautions, which includes use of gloves, gown, mask, and eye protection. For more information on Universal Precautions, see www.osha.gov/SLTC/bloodbornepathogens/index.html#revised_standard.
- Any procedure with the potential to generate fine-particulate aerosols (e.g., vortexing or sonication of specimens in an open tube) should be performed in a biological safety cabinet (BSC). Use sealed centrifuge rotors or sample cups, if available, for centrifugation. Ideally, rotors and cups should be loaded and unloaded in a BSC. Perform any procedures outside a BSC in a manner that minimizes the risk of exposure to an inadvertent sample release.

Supplement F: Laboratory Guidance
(continued from previous page)

- After specimens are processed, decontaminate work surfaces and equipment. Use any EPA-registered hospital disinfectant. Follow manufacturer's recommendations for use-dilution (i.e., concentration), contact time, and care in handling.

B. Other specimens (e.g., respiratory secretions, stool, or tissue for procedures performed in microbiology or pathology laboratories)

- The following activities may be performed in BSL-2 facilities with standard BSL-2 work practices:

  - Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues
  - Molecular analysis of extracted nucleic acid preparations
  - Electron microscopic studies with glutaraldehyde-fixed grids
  - Routine examination of bacterial and mycotic cultures
  - Routine staining and microscopic analysis of fixed smears
  - Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.

- The following activities involving manipulation of untreated specimens should be performed in BSL-2 facilities and in a Class II BSC:

  - Aliquoting and/or diluting specimens
  - Inoculating bacterial or mycological culture media
  - Performing diagnostic tests that do not involve propagation of viral agents in vitro or in vivo
  - Nucleic acid extraction procedures involving untreated specimens
  - Preparation and chemical- or heat-fixing of smears for microscopic analysis

  Work surfaces should be decontaminated on completion of work with appropriate disinfectants. All disposable waste should be autoclaved.

  Laboratory workers should wear personal protective equipment (PPE), including disposable gloves and laboratory coats.

  Any procedure or process that cannot be conducted in a BSC should be performed while wearing gloves, gown, eye protection, and respiratory protection, (see Supplement I, Section III.D.5).

  Acceptable methods of respiratory protection include: a properly fit-tested, NIOSH-approved filter respirator (N-95 or higher level) or a powered air-purifying respirator (PAPR) equipped with high-efficiency particulate air (HEPA) filters. Accurate fit-testing is a key component of effective respirator use.[1] Personnel who cannot wear fitted respirators because of facial hair or other fit limitations should wear loose-fitting hooded or helmeted PAPRs.

  Appropriate physical containment devices (e.g., centrifuge safety cups; sealed rotors) should also be used. Ideally, rotors and cups should be loaded and unloaded in a BSC.

[1] Respirators should be used in the context of a complete respiratory protection program, as required by the Occupational Safety and Health Administration (OSHA). This includes training, fit-testing, and fit-checking to ensure appropriate respiratory selection and use. To be effective, respirators must provide a proper sealing surface on the wearer's face. Detailed information on a respiratory protection program can be found at: www.osha.gov/SLTC/etools/respiratory/.

Supplement F: Laboratory Guidance
(continued from previous page)

- The following activities must be performed in a BSL-3 facility using BSL-3 work practices:

  - SARS-CoV propagation in cell culture
  - Initial characterization of viral agents recovered in cultures of SARS specimens

  Any procedure or process that cannot be conducted in a BSC should be performed while wearing gloves, gown, eye protection, and respiratory protection (see Supplement I, Section III.D.5).

  Acceptable methods of respiratory protection include: a properly fit-tested, NIOSH-approved filter respirator (N-95 or higher level) or PAPR equipped with HEPA filters. Accurate fit-testing is a key component of effective respirator use.[1] Personnel who cannot wear fitted respirators because of facial hair or other fit limitations should wear loose-fitting hooded or helmeted PAPRs.

  Centrifugation should be carried out using sealed centrifuge cups or rotors that are unloaded in a BSC.

- The following activities must be performed in Animal BSL-3 facilities using Animal BSL-3 work practices:

  - Inoculation of animals for potential recovery of SARS-CoV from SARS samples
  - Protocols involving animal inoculation for characterization of putative SARS agents

Consideration may also be given to referral of specimens to a suitably equipped reference laboratory.

Note: Packaging, shipping, and transport of specimens from possible and known cases of SARS-CoV disease must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations at (www.iata.org/dangerousgoods/index) and US DOT 49 CFR Parts 171-180 (hazmat.dot.gov/rules.htm). Step-by-step instructions on appropriate packaging and labeling are provided at: www.cdc.gov/ncidod/sars/pdf/packingspecimens-sars.pdf

[1] Respirators should be used in the context of a complete respiratory protection program, as required by the Occupational Safety and Health Administration (OSHA). This includes training, fit-testing, and fit-checking to ensure appropriate respiratory selection and use. To be effective, respirators must provide a proper sealing surface on the wearer's face. Detailed information on a respiratory protection program can be found at: www.osha.gov/SLTC/etools/respiratory/.

(continued from previous page)

Appendix F6
Guidelines for Medical Surveillance of Laboratory Personnel Working with SARS-CoV

Key Messages

- Laboratory workers should receive training on the appropriate biosafety level for the type of work being performed.
- Before working with either live SARS-CoV or clinical specimens known to contain SARS-CoV, laboratory workers should have a baseline serum sample obtained and stored for future reference.
- Laboratory workers in laboratories that contain live SARS-CoV should report any fever or lower respiratory symptoms to their supervisor. They should be evaluated for possible exposures, and the clinical features and course of their illness should be closely monitored.
- Laboratory workers who are believed to have had a laboratory exposure to SARS-CoV should be evaluated, counseled about the risk of SARS-CoV transmission to others, and monitored for fever or lower respiratory symptoms as well as for any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, diarrhea.
- Local and/or state public health departments should be promptly notified of laboratory exposures and illness in exposed laboratory workers.

Medical surveillance of laboratory personnel can help to ensure that workers who are at risk of occupational exposure to SARS-CoV and who develop symptoms of illness receive appropriate medical evaluation and treatment, both for the benefit of their health and to prevent further transmission.

- Laboratory workers should be provided training on the appropriate biosafety level and specific safety practices for the type of work being performed. Biosafety guidelines for laboratory work with SARS-CoV are available at: www.cdc.gov/ncidod/sars/sarslabguide.htm.
- Before working with either live SARS-CoV or clinical specimens known to contain SARS-CoV, laboratory workers should have a baseline serum sample obtained and stored for future reference.
- Laboratory workers should immediately contact their supervisor in the event of a recognized exposure or development of lower respiratory symptoms and/or fever. In addition, exposed laboratory workers should be monitored for the presence of any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, diarrhea. The supervisor should immediately contact appropriate healthcare personnel and facility contacts (e.g., occupational health, infection control, or a designee); the local and/or state public health departments should be promptly notified as well.

I. Management of a Break in Laboratory Procedure

In the event of an identifiable break in laboratory procedure (e.g., tear in a glove; spill of live virus), the laboratory worker should immediately implement applicable laboratory procedures for emergency

(continued from previous page)

exposure management and/or environmental decontamination and notify the supervisor for further instructions. The worker and the supervisor, in consultation with occupational health or infection control personnel, should evaluate the break in procedure to determine if an exposure occurred. If the break in procedure resulted in an exposure, the worker should be managed as described below.

II. Management of Exposed Laboratory Workers

A. Management of exposed laboratory workers who are asymptomatic

1. Exposed workers should be instructed to be vigilant for the development of fever (i.e., measure and record body temperature twice daily for 10 days after the date of the last unprotected exposure), lower respiratory symptoms, or any of the following: sore throat, rhinorrhea, chills, rigors, myalgia, headache, diarrhea. Exposed workers should immediately notify the supervisor if symptoms develop.

2. Exposed workers should be actively monitored for symptoms prior to reporting for duty.

3. Decisions regarding activity restrictions (e.g., work) should be discussed with the health department, in accordance with the recommendations in Supplement D. Asmptomatic exposed workers generally do not need to be excluded from duty. However, a worker who has had a high-risk exposure may need to be furloughed.

B. Management of exposed laboratory workers who develop symptoms within 10 days of exposure

1. The exposed laboratory worker who develops fever, lower respiratory symptoms, sore throat, rhinorrhea, chills, rigors, myalgia, headache, or diarrhea should:

   - Immediately put on a surgical mask if at work, *and*
   - Immediately notify the appropriate facility contact (e.g., infection control, occupational health, or a designee in each facility where s/he works), *and*
   - Report to the designated location for clinical evaluation.

2. Symptomatic laboratory workers should be managed in accordance with the recommendations in *Clinical Guidance on the Identification and Evaluation of Possible SARS-CoV Disease among Persons Presenting with Community-Acquired Illness* www.cdc.gov/ncidod/sars/clinicalguidance.htm.

3. Decisions on return to work should be guided by policies or regulations defined by the facility or health department.

III. Management of Symptomatic Laboratory Workers with No Recognized Exposures

Laboratory workers who develop a fever or lower respiratory symptoms and who have no recognized exposure should immediately contact the supervisor. The supervisor should immediately contact the appropriate healthcare personnel and facility contacts (e.g., occupational health, infection control, or a designee), who should review the worker's illness and potential laboratory exposures to determine if any SARS precautions or additional consultations are necessary. If clinical or exposure information suggests SARS-CoV infection, local or state public health officials should be immediately be contacted and consulted about managing the ill laboratory worker and contacts.

(continued from previous page)

Appendix F7
Fact Sheet for Clinicians: Interpreting SARS-CoV Test Results from CDC and Other Public Health Laboratories

Key Messages

- A positive RT-PCR test result for SARS-CoV should be considered presumptive until confirmatory testing by a second reference laboratory is performed.
- A negative test result for SARS-CoV may not rule out SARS-CoV disease and should not affect patient management or infection control decisions.

Definitions

SARS	Severe acute respiratory syndrome
SARS-CoV	SARS-associated coronavirus; a newly described coronavirus that is genetically and antigenically distinct from other human coronaviruses
Laboratory-confirmed SARS-CoV infection	• Detection of any of the following by a validated test, with confirmation in a reference laboratory: o Serum antibodies to SARS-CoV in a single serum specimen, *or* o A four-fold or greater increase in SARS-CoV antibody titer between acute- and convalescent-phase serum specimens tested in parallel, *or* o Negative SARS-CoV antibody test result on acute-phase serum and positive SARS-CoV antibody test result on convalescent-phase serum tested in parallel; **or** • Isolation in cell culture of SARS-CoV from a clinical specimen, with confirmation using a test validated by CDC; **or** • Detection of SARS-CoV RNA by RT-PCR validated by CDC, with confirmation in a reference laboratory, from: o Two clinical specimens from different sources, *or* o Two clinical specimens collected from the same source on two different days
Confirmed case of SARS-CoV disease	A person with clinically compatible illness and laboratory-confirmed SARS-CoV infection

Supplement F: Laboratory Guidance
(continued from previous page)

The Centers for Disease Control and Prevention (CDC) and other institutions have been working to develop strategies to detect and control the spread of severe acute respiratory syndrome (SARS). The cause of SARS has been determined to be infection with a previously unrecognized human coronavirus, SARS-associated coronavirus (SARS-CoV). Information on SARS and SARS-CoV is provided on CDC's SARS website: www.cdc.gov/sars/. All information and guidelines, including those on SARS-CoV laboratory testing, may change as we continue to learn more about this disease. Please check CDC's SARS website regularly for the most current information.

Previous experience with SARS-CoV disease demonstrates that the best guide to diagnosis is exposure to a person with SARS-CoV disease, a setting where SARS-CoV transmission is occurring, or persons who are part of a cluster of pneumonia without a known cause. Information in diagnosing SARS-CoV disease is provided in *Clinical Guidance on the Identification and Evaluation of Possible SARS-CoV Disease among Persons Presenting with Community-Acquired Illness* (www.cdc.gov/ncidod/sars/clinicalguidance.htm). Persons without a potential risk of exposure should usually not be tested for SARS-CoV. Clinicians should seek guidance from the state or local health department regarding current guidelines for SARS-CoV testing.

Clinicians providing care for patients with possible SARS-CoV disease may find the following information useful when interpreting SARS-CoV test results.

What tests for SARS-CoV are available?
CDC has developed and validated an enzyme immunoassay (EIA) for detection of serum antibody (www.cdc.gov/ncidod/sars/lab/eia/) to SARS-CoV and a reverse transcription-polymerase chain reaction (RT-PCR) assay (www.cdc.gov/ncidod/sars/lab/rtpcr/) for detection of SARS-CoV RNA. The EIA has been distributed to most state public health laboratories, and the RT-PCR has been distributed to most laboratories in the Laboratory Response Network (LRN). Both the EIA and the RT-PCR tests are sensitive and highly specific for SARS-CoV. The ability to diagnose SARS-CoV infection in a patient is often limited, however, by either the low concentration of virus in most clinical specimens (RT-PCR assays) or the time it takes a person to mount a measurable antibody response to SARS-CoV (serologic assays). The likelihood of detecting infection is increased if multiple specimens (e.g., stool, serum, respiratory tract specimens) are collected at several times during the course of illness.

CDC considers detection of SARS-CoV antibody to be the most reliable indicator of infection. Since previous infection is still rare in most populations, seroconversion is not needed to diagnose infection. Therefore, the presence of SARS-CoV antibody in someone without a previous history of SARS is indicative of recent infection. A negative serologic test can rule out SARS-CoV infection if the serum specimen is collected >28 days after onset of illness. Some persons do not mount an antibody response (test positive) until more than 28 days after onset of illness. Patients with a negative antibody test result whose specimens were obtained 28 days before illness onset or before should have another serum specimen collected >28 days after onset of symptoms.

RT-PCR for SARS-CoV RNA is a very sensitive and specific assay when performed appropriately. This test can detect SARS-CoV RNA in serum, stool, upper and lower respiratory specimens, various tissues, and occasionally urine specimens. Testing of multiple specimen types at several times during the course of illness should increase the likelihood of detecting infection.

Other tests for detection of SARS-CoV include immunofluorescence assay (IFA) for SARS-CoV antibody, SARS-CoV isolation studies, electron microscopic studies, and immunohistologic or in situ hybridization studies on tissue specimens. The IFA for SARS-CoV antibody gives results essentially identical to those

Supplement F: Laboratory Guidance
(continued from previous page)

for the EIA for SARS antibody. Cell culture, electron microscopy, and histologic studies are less frequently used and less sensitive than RT-PCR. Cell culture for SARS-CoV should be done only in a BSL-3 laboratory using BSL-3 procedures (see Appendix F5).

What does it mean if a specimen tests positive for SARS-CoV?
Laboratory test results should always be considered with clinical observations and epidemiologic data in making a final diagnosis. A positive RT-PCR result should be confirmed by testing a second specimen and confirming the result at a qualified second laboratory to ensure that the result is not an artifact of laboratory contamination. A positive serologic result is less likely to result from a laboratory artifact but should also be subjected to confirmatory testing. If the results are confirmed, then a positive RT-PCR or serologic test result indicates that the patient has been recently infected with SARS-CoV (unless the patient has a previous history of SARS-CoV disease). Guidelines for managing patients with SARS-CoV disease are provided in Supplement C and Supplement I.

How is a SARS-CoV test confirmed?
Positive antibody and RT-PCR test results should be confirmed by repeat testing of the original specimen AND by testing of the same specimen in an independent laboratory using a validated assay.

What is difference between a laboratory-confirmed clinical specimen and laboratory-confirmed SARS-CoV disease?
This distinction is made for PCR test results because of concerns about false-positive results. For serology, virus isolation, and histopathologic studies, if a specimen is confirmed positive, the patient is also considered to be confirmed positive. For PCR, a second specimen is required to be confirmed positive to decrease the chance of misclassifying a patient due to a false-positive result. In all instances, laboratory results must be considered in the context of clinical and epidemiologic information on the patient.

What does it mean if a patient with an illness suggestive of SARS has a negative SARS-CoV test result?
A negative antibody result on a serum specimen collected >28 days after onset of illness is sufficient to eliminate SARS-CoV as the cause of illness. A negative antibody result on serum specimens collected ≤28 days after onset of illness or a negative RT-PCR test does not rule out SARS-CoV infection. Clinical specimens do not always have sufficient virus to be detected by RT-PCR. An antibody response may not be detected in some patients until >28 days after onset of illness.

What does it mean if test results are positive for other respiratory diseases?
A positive test result for another respiratory pathogen does not rule out SARS-CoV disease. SARS patients can be co-infected with SARS-CoV and other respiratory pathogens. Thus, detection of another respiratory pathogen does not eliminate the possibility of SARS-CoV disease. In some circumstances (e.g., another pathogen is detected in multiple patients in a cluster of cases and can fully explain the severity of illness), detection of another respiratory pathogen may make SARS-CoV disease less likely. Factors that may be considered in assigning alternate diagnoses include the strength of the epidemiologic exposure criteria for SARS-CoV disease, the specificity of the diagnostic test, and the compatibility of the clinical presentation and course of illness with the alternative diagnosis.

Does a negative SARS-CoV test result affect patient management?
As noted above, the interpretation of negative SARS-CoV test results varies depending on the type of specimen, the timing of specimen collection, and the test that was performed. With the exception of a >28-day negative serologic test result, a negative SARS-CoV test result should not affect patient isolation or management decisions. The clinical features of the illness and the type and risk of exposure are the keys to making decisions on patient management and isolation.

Supplement F: Laboratory Guidance
(continued from previous page)

Has the identification of SARS-CoV as the etiologic agent changed the recommendations for medical treatment of patients with SARS?
No. The discovery that SARS-CoV is the cause of SARS has not changed treatment recommendations. Research on antiviral treatment for SARS-CoV disease is currently under way.

Should a person who may have been exposed to a location with transmission of SARS-CoV or who had contact with a SARS patient be tested even if not ill?
Persons who have potentially been exposed to SARS patients and are well should be tested only as part of research studies. The exposed person may contact their state health department or CDC about participating in studies of persons exposed to SARS-CoV.

Supplement F: Laboratory Guidance
(continued from previous page)

Appendix F8
Guidelines for Laboratory Diagnosis of SARS-CoV Infection

Laboratory confirmation of SARS-CoV infection is based on:

- Detection of any of the following by a validated test, with confirmation in a reference laboratory:
  - Serum antibodies to SARS-CoV in a single serum specimen, *or*
  - A four-fold or greater increase in SARS-CoV antibody titer between acute- and convalescent-phase serum specimens tested in parallel, *or*
  - Negative SARS-CoV antibody test result on acute-phase serum and positive SARS-CoV antibody test result on convalescent-phase serum tested in parallel; **or**

- Isolation in cell culture of SARS-CoV from a clinical specimen, with confirmation using a test validated by CDC; **or**

- Detection of SARS-CoV RNA by RT-PCR validated by CDC, with confirmation in a reference laboratory, from:
  - Two clinical specimens from different sources, *or*
  - Two clinical specimens collected from the same source on two different days

Guidelines for the collection of specimens from potential cases of SARS are provided in Appendix F4.